

Oxygen Yield and Thermoluminescence Characteristics of a Cyanobacterium Lacking the Manganese-Stabilizing Protein of Photosystem II†

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ABSTRACT: Previous experiments have shown that a *Synechocystis* sp. PCC 6803 mutant ($\Delta psbO$) lacking the extrinsic manganese-stabilizing protein (MSP) exhibits impaired, but significant levels of H_2O -splitting activity [Burnap, R., & Sherman, L. A. (1991) *Biochemistry* 30, 440-446]. [^{14}C]DCMU-binding experiments now show that the number and affinity of DCMU-binding sites (normalized to chlorophyll) are equivalent in $\Delta psbO$ and the wild type, suggesting equal concentrations of assembled reaction centers. A similar conclusion is reached on the basis of measurements of PSII electron transport (DPC-supported DCPIP reduction) by mutant and wild-type thylakoids. The pattern of flash O_2 yield by $\Delta psbO$ cells measured with a bare platinum electrode exhibits a period four oscillation (with a maximum on the third flash), indicating that the H_2O -splitting enzyme in $\Delta psbO$ retains the basic mechanistic features found in normal cells. However, the amplitude of these signals is smaller and more highly damped than those obtained from wild-type cells, suggesting the absence of MSP results in a higher miss probability and/or a reduction in the number of centers competent in oxygen evolution. Analysis of the rise kinetics of the amperometric signal on the bare platinum electrode indicates that the S_3 -[S_4]- S_0 transition is retarded by at least a factor of 5 in the mutant. Thermoluminescence emission peak temperatures indicate that the $S_2Q_A^-$, $S_2Q_B^-$, and $S_3Q_B^-$ charge pairs are significantly more stable with respect to recombination in the mutant. The intensities of the thermoluminescence emissions are also significantly reduced in the mutant. Taken together, the data suggest that functional consequences of the genetic removal of MSP are complex. Although the number of photochemically active PSII reaction centers is not much changed by the absence of MSP, the proportion of centers which are coupled to functional O_2 -evolving enzymes appears to be reduced. For those centers which are effectively coupled to O_2 evolution, we find evidence of alterations in the kinetic properties of the enzyme due to the absence of MSP. These are (1) an increased miss factor, (2) a retardation of the S_3 -[S_4]- S_0 transition, and (3) an increase in the stabilization of the S_2 and S_3 states.

The H_2O -splitting reaction of oxygenic photosynthesis is catalyzed by the membrane-bound photosystem II (PSII)¹ complex. The oxidative decomposition of two molecules of H_2O involves the utilization of four oxidizing equivalents accumulated in the H_2O -splitting enzyme as a result of four successive charge-separation and -transfer events within the PSII reaction center. Accordingly, the H_2O -splitting enzyme passes through a series of oxidation states, termed S states, which correspond, at least in part, to the stepwise oxidation

of a cluster of four Mn atoms believed to form the catalytic center of the H_2O -splitting reaction [For recent reviews on PSII, see Babcock et al. (1989), Bruvig, et al. (1989), Ghanotakis and Yocum (1990), and Hansson and Wydrzynski (1990)].

The structure of the protein environment forming the active site of the H_2O -splitting enzyme remains to be elucidated. Current evidence suggests that the atoms of the Mn cluster are ligated by one or more of the intrinsic polypeptides of the PSII complex in a region accessible to the aqueous space of the thylakoid lumen (Andersson et al., 1987; Seibert et al., 1989; Mei et al., 1989; Svensson et al. 1990). In addition to the intrinsic PSII polypeptides, one or more extrinsic polypeptides are associated with the H_2O -splitting portion of the PSII complex [see Ghanotakis and Yocum (1990)]. Three such polypeptides with approximate molecular weights of 33 000, 24 000, and 18 000 have been identified in higher plants and eukaryotic algae. Cyanobacteria appear to lack homologs for the 24- and 18-kDa extrinsic polypeptides, while a homolog for the 33-kDa extrinsic polypeptide has been found in all species examined to date.

The 33-kDa extrinsic polypeptide has been referred to as the Mn-stabilizing protein (MSP) on the basis of biochemical depletion/reconstitution experiments showing that it stabilizes the binding of two of the four active site Mn atoms (Ono & Inoue, 1984). The loss of active site Mn, following biochemical removal of MSP, can be suppressed by the presence of high concentrations (>100 mM) of Cl^- (Kuwabara et al., 1985).

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Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, 2-(*N*-morpholino)ethanesulfonic acid; MSP, manganese-stabilizing protein, extrinsic 33-kDa PSII protein; Q_A , primary plastoquinone electron acceptor; Q_B , secondary, exchangeable plastoquinone electron acceptor; P680, photooxidizable chlorophyll species acting as primary electron donor of the reaction center; PSII, photosystem II; *psbO*, gene encoding the manganese-stabilizing protein; S_n , oxidation states of the H_2O -splitting enzyme, where n represents the number of stored oxidizing equivalents; Y_Z , redox-active tyrosine of the D1 protein acting as secondary electron donor of the reaction center.